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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<p>(21) International Application Number: PCT/AU96/00258</p> <p>(22) International Filing Date: 2 May 1996 (02.05.96)</p> <p>(30) Priority Data: PN 2742 2 May 1995 (02.05.95) AU</p> <p>(71) Applicant (for all designated States except US): GARVAN INSTITUTE OF MEDICAL RESEARCH [AU/AU]; c/o St. Vincents Hospital, 384 Victoria Street, Darlinghurst, NSW 2010 (AU).</p> <p>(72) Inventors; and (75) Inventors/Applicants (for US only): DALY, Roger, John [GB/AU]; 49 Gerard Street, Alexandria, NSW 2015 (AU). SUTHERLAND, Robert, Lyndsay [AU/AU]; 20 Northcote Road, Lindfield, NSW 2070 (AU).</p> <p>(74) Agent: F.B. RICE & CO.; 28A Montague Street, Balmain, NSW 2041 (AU).</p>	<p>(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>With international search report.</i></p>	
<p>(54) Title: GDU, A NOVEL SIGNALLING PROTEIN</p>		
<p>(57) Abstract</p> <p>The present invention provides the nucleotide and amino acid sequence of a previously unidentified erbB receptor target. The nucleotide and amino acid sequence is set out in the figure.</p>		

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GDU, A Novel Signalling Protein

The present invention relates to a previously unidentified erbB receptor target designated GDU. The present invention relates to a polynucleotide encoding GDU and to methods of detecting the presence of GDU.

Many intracellular targets for receptor tyrosine kinases (RTKs) contain one or more src homology (SH)2 domains. These are conserved, non-catalytic domains of approximately 100 amino acids which bind to short peptide sequences containing phosphotyrosine (Cohen *et al*, Cell 80, 237-248, 1995). Since receptor autophosphorylation on specific tyrosine residues follows RTK activation, SH2 domains mediate receptor-substrate, as well as other protein-protein interactions, during signal transduction. SH2 domains contain not only a pocket lined with basic residues which binds the phosphotyrosine but also an additional binding pocket or groove which interacts with amino acids C-terminal to this residue, this determining the specificity of the interaction. The particular autophosphorylation sites present on a given RTK therefore define the SH2 domain-containing signalling proteins that it can recruit and hence, to a large extent, the signalling specificity of the receptor. SH2 domains are often accompanied in signalling proteins by two other conserved protein modules; SH3 domains, which bind to proline-rich peptide ligands, and pleckstrin-homology (PH) domains. The function of the latter remains ill-defined, and both protein and phospholipid ligands have been described.

SH2 domain-containing proteins can be divided into two classes (Schlessinger and Ullrich Neuron, 9,383-301 1992); Class I, which also possess a catalytic function e.g. phospholipase C- γ 1 (PLC- γ 1) and the GTPase activating protein for Ras (Ras-GAP), and Class II, which contain only non-catalytic protein modules and are thought to function as adaptors, linking separate catalytic subunits to receptors or other signalling proteins e.g. Grb2. The tissue expression of particular SH2 domain-containing proteins varies from ubiquitous, e.g. Grb2, which performs a fundamental role in linking tyrosine kinases to Ras signalling, to relatively restricted e.g. Grb7, which is mainly expressed in the liver and kidney (Margolis *et al* Proc. Natl. Acad. Sci. USA, 89, 8894-8898, 1992). Presumably the latter protein performs relatively specialised signalling functions. The CORT (cloning of receptor targets) technique, in which cDNA expression libraries are screened with the

tyrosine phosphorylated C-terminus of the EGF receptor represents a powerful methodology for the identification and characterisation of novel, SH2 domain-containing, receptor substrates (Skolnik et al Cell 65, 83-90, 1991).

5 Members of the *erbB* family of RTKs and their ligands are implicated both in normal mammary gland development and the growth and progression of human breast cancer. Furthermore, marked alterations in the expression or activity of several SH2 domain-containing proteins have been observed in human breast cancers or breast cancer-derived cell lines,
10 suggesting that this represents an additional level at which RTK signalling may be modulated in this disease (Daly, Breast Cancer Res Treat, 34, 85-92, 1995). We therefore chose normal human mammary epithelial cells as a basis for a CORT screening program and hence identification of novel, and relatively tissue specific, *erbB* receptor targets.

15 Screening of a HMEC 184 λ EXlox cDNA library isolated 1 Ras-GAP, 2 Grb2 cDNAs and a cDNA encoding a novel SH2 domain-containing protein. This protein, designated GDU or Grb14 (the designations "GDU" and "Grb14" are used interchangeably herein), is related both in molecular architecture and sequence homology to Grb7 and Grb10, previously
20 identified *erbB* receptor targets. These three proteins also share significant sequence homology, over an approximately 300 amino acid region encompassing the PH domain, with the *C. elegans* gene F10E9.6. The latter gene has recently been shown to encode a protein (mig 10) critical for longitudinal neuronal migration in *C. elegans*; members of the Grb7 gene
25 family, including GDU, may therefore be involved in the regulation of cell migration in higher organisms.

 Analysis of GDU gene expression in normal breast epithelial cells and a large series of human breast cancer cell lines revealed that expression was limited predominantly to normal breast cells and the more highly
30 differentiated, estrogen receptor positive, breast cancer cell lines. Also, GDU mRNA was overexpressed in the DU-145 prostate carcinoma cell line relative to the normal prostate and two other prostate cancer cell lines. GDU may therefore serve as a prognostic indicator and/or a tumour marker in both breast and prostate cancer. Furthermore, since altered expression of GDU
35 may contribute to the abnormal proliferation, invasion and/or migration of cancer cells, GDU signal transduction may provide a novel therapeutic target

in human cancer. Finally, since GDU is involved in downstream signalling initiated by the platelet derived growth factor receptor (PDGFR), it may provide a target in diseases or conditions in which PDGF plays a regulatory role e.g. wound healing, fibrotic conditions, atherosclerosis.

5 In a first aspect the present invention consists in a polynucleotide encoding GDU, the polynucleotide having a sequence which encodes a polypeptide having an amino acid sequence as shown in Figure 2 or a sequence which hybridises thereto.

10 In a preferred embodiment of the present invention the polynucleotide has a sequence as shown in Figure 2.

In a second aspect the present invention consists in a polypeptide, the polypeptide having an amino acid sequence as shown in Figure 2.

In a third aspect the present invention consists in an antibody which binds to the polypeptide of the second aspect of the present invention.

15 The antibody may be monoclonal or polyclonal, however, it is presently preferred that the antibody is a monoclonal antibody.

In a fourth aspect, the present invention consists in an oligonucleotide probe of at least 12 nucleotides, the oligonucleotide probe having a sequence such that the probe selectively hybridises to the polynucleotide of the first aspect of the present invention under stringent conditions.

In a preferred embodiment of this aspect of the present invention the oligonucleotide is labelled. In a further preferred embodiment of the present invention the oligonucleotide is of at least 18 nucleotides.

25 In a fifth aspect the present invention consists in method of detecting the presence of GDU in a sample, the method comprising reacting the sample with an antibody of the second aspect of the present invention or a oligonucleotide probe of the fourth aspect of the present invention and detecting the binding of the antibody or the probe.

30 In order that the nature of the present invention may be more clearly understood preferred forms thereof will now be described with reference to the following examples and Figures in which:-

35 **Figure 1** shows a schematic representation of Grb14 structure with a restriction map for the Grb14 cDNA and the cDNA clones used to derive the Grb14 sequence aligned underneath. The initial clone isolated by CORT screening was designated clone 1. Two other clones (1-1 and 1-2) were

isolated from the 184 cell line library by screening using clone 1 as a probe. The Grb14 cDNA sequence was completed using two clones L5 and L6, isolated from a human liver cDNA library. Abbreviations are as follows: A; Apa I; Av; Avr II, X; Xho I; E; Eco RI. The numbers refer to distance in bp.

5 **Figure 2** shows the nucleotide and amino acid sequence of Grb14. The PH domain is underlined and the SH2 domain indicated by bold type. The translation termination codon is shown by an asterisk in the amino acid sequence. Numbers refer to distances in bp.

10 **Figure 3** shows the sequence homology between Grb14, Grb7, Grb10 and F10E9.6. As alignment of the amino acid sequences of Grb14, mouse Grb7, mouse Grb10 and *C. elegans* F10E9.6 was obtained using the computer programs Clustal W and SeqVu. Identical residues are boxed. A highly conserved proline-rich motif is indicated by the dotted underline, the PH domain by the broken underline and the SH2 domain by the bold underline.

15 Only the central region of F10E9.6 exhibiting homology with the Grb7 family is shown. Amino acid residues for each protein are numbered (from the initiation methionine) on the right.

20 **Screening of a Normal Breast Epithelial Cell cDNA Library by the CORT Technique**

CORT screening of two cDNA libraries prepared from normal breast epithelial cells led to the isolation of recombinants which exhibited differential binding to the phosphorylated EGFR C-terminus. Upon excision of the corresponding pEXlox plasmids and sequencing of the DNA inserts,

25 two recombinants which bound very strongly were identified as Grb2 cDNA clones (Lowenstein *et al* 1992, Cell 70, 431-442, 1992), and a clone exhibiting moderate binding corresponded to ras-GAP (Trahey *et al.* Science 242, 1696-1700, 1988). The final clone, designated GDU, bound only weakly to the EGFR. A database search with the corresponding cDNA sequence did not

30 detect an exact match but revealed significant sequence homology with the SH2 domain-containing protein Grb7 (Margolis *et al* PNAS 89, 8894-8898, 1992). The cDNA (GDU Clone 1 in Figure 1) encoded a short stretch of amino acids followed by a C-terminal SH2 domain; homology to Grb7 was apparent over this entire open reading frame.

35

Characterisation of GDU

In order to obtain the full length cDNA sequence for GDU, two cDNA library screens were performed. In the first, the cDNA insert from Clone 1 was used to screen the breast cDNA library. Screening of 5×10^5 recombinants isolated 2 cDNAs, designated 1-1 and 1-2, of 1.6 and 1.4kb, respectively (Figure 1). In the second, a 213bp EcoRI-Xho I restriction fragment derived from 1-1 (Figure 1) was used to screen a human liver cDNA library. Screening of 1×10^6 recombinants isolated 2 cDNAs, designated L5 and L6, of 1.3 and 1.7kb, respectively (Figure 1). Clones 1-1, 1-2, L5 and L6 were sequenced in their entirety on both strands to obtain the cDNA sequence shown in Figure 2. The 2.4kb of DNA sequence derived from these overlapping clones corresponds closely to the size of the three most abundant mRNA species detected upon Northern blot analysis.

Analysis of the cDNA sequence identified an open reading frame of 540 amino acids. The initiation codon is preceded by an in-frame termination codon and is surrounded by a consensus sequence for strong translational initiation. The encoded protein is similar both in molecular architecture and amino acid sequence to Grb7 (Margolis et al, Proc. Natl. Acad. Sci. USA **89**, 8894-8898, 1992) and the recently identified Grb10 (Ooi et al, Oncogene **10**, 1621-1630, 1995), consisting of a N-terminal region containing at least one proline-rich motif, a central region which exhibits significant homology to the putative *C. elegans* protein F10E9.6 (Stein et al EMBO J, **13**, 1331-1340, 1994) and which also encompasses a PH domain, and a C-terminal SH2 domain. An alignment of the amino acid sequences of GDU, Grb7, Grb10 and F10E9.6 is shown in Figure 3.

GDU is similar in size to Grb7, Grb10 possessing a more extended N-terminus. The N-terminal region exhibits low sequence homology between GDU, Grb7 and Grb10 apart from a highly conserved amino acid motif PS/AIPNPFPEL. Also of note is the presence of two clusters of basic residues which flank this motif. Overall the N-terminal region of GDU displays a lower proline content than that of Grb7 and Grb10 (GDU amino acids 1-110; 11% proline, Grb10 amino acids 1-113; 15%, Grb7 amino acids 1-103; 23%).

GDU, Grb7 and Grb10 share a central, conserved region of approximately 320 amino acids which exhibits significant homology to a domain found in the *C. elegans* protein F10E9.6. Over this region, GDU

bears 48, 55 and 28% amino acid identity respectively with Grb7, Grb10 and F10E9.6 (Figure 3). The core of this region is provided by a PH domain (Figures 1, 2 and 3), over which GDU exhibits 56, 61 and 35% amino acid identity, respectively, with Grb7, Grb10 and F10E9.6. However, as noted by
5 Ooi et al, (Oncogene 10, 1621-1630, 1995) another region of particularly marked homology spanning approximately 100 amino acids exists amino-terminal to the PH domain (Figure 3).

The most highly conserved region amongst Grb7 family members is the SH2 domain (Figure 3). The GDU SH2 domain displays 67 and 74%
10 amino acid identity, respectively, with the corresponding domain in Grb7 and Grb10.

Northern Blot Analysis of GDU Gene Expression

The tissue specificity of GDU gene expression was investigated by hybridizing Northern blots of poly A⁺ RNA isolated from a variety of human
15 tissues to a GDU specific cDNA probe. GDU gene expression was highest in the testis, ovary, heart, liver, skeletal muscle, kidney and pancreas. Moderate expression was detected in the small intestine, colon, peripheral blood leukocytes, brain and placenta, whilst expression in the spleen, thymus, prostate and lung was low or undetectable. Several mRNA
20 transcripts were detected which displayed tissue-specific variation in their relative abundance. The three most prominent transcripts were approximately 2.3, 2.4 and 2.5kb. Often co-expressed with one or two of these transcripts was a transcript of approximately 9.5kb. In the ovary a still larger transcript of undetermined size was also expressed.

25 Since the Grb14 cDNA was originally isolated from a cDNA library prepared from normal human breast epithelial cells, we were interested in determining the expression profile of Grb14 mRNA in a panel of human breast cancer cell lines. Upon Northern blot analysis of total RNA isolated from 3 normal human breast epithelial cell strains and 19 human breast
30 cancer cell lines, Grb14 gene expression could be detected in HMEC 184 and HMEC-219-4 cells, 6/7 ER+ human breast cancer cell lines and 2/12 ER- cell lines (Table 1). Thus Grb14 gene expression appears largely restricted to normal breast epithelial and ER+ breast cancer cells. Differential expression of Grb14 was also observed amongst human prostate cancer cell lines.
35 Although Grb14 mRNA expression was undetectable in the normal prostate,

low expression could be detected in the PC3 and LnCaP prostate cancer cell lines and high expression in the DU145 line (Table 1).

Origin	Cell Line	Expression
Normal human breast epithelial	HMEC 184	+++
	HMEC-219-4	+
	HMEC-1001-7	-
Human breast cancer, ER+	T-47D	+++
	ZR-75-1	++
	MCF-7	+
	BT-483	+
	MDA-MB-134	+
	MDA-MB-361	+
	BT-474	-
Human breast cancer, ER-	MDA-MB-330	+
	MDA-MB-468	+
	BT-20	-
	SK-BR-3	-
	BT-549	-
	H3578T	-
	DU-4475	-
	MDA-MB-157	-
	MDA-MB-175	-
	MDA-MB-231	-
	MDA-MB-436	-
	MDA-MB-453	-
Human prostate cancer	PC3	+
	LnCaP	+
	DU-145	++++
Human epidermoid carcinoma	A431	-
Human embryonic kidney	HEK 293	++++

- 5 **Table 1.** Expression of Grb14 mRNA in different human cell lines. Total cellular RNA was extracted from the indicated cell lines and subjected to Northern blot analysis using a Grb14 cDNA probe. The relative expression levels of Grb14 mRNA were then scored on a scale from + (low) to ++++ (high). -; undetectable expression.

Expression of Grb14 Protein

In order to characterize the Grb14 protein a polyclonal antiserum was raised against a GST-Grb14 SH2 domain fusion protein. Following
5 affinity purification, this antiserum was used to Western blot cell lysates derived from cell lines in which Grb14 mRNA was either expressed at high levels (DU145 and HEK 293) or was undetectable (A431 and SK-BR-3) (Table 1). The antiserum recognized a protein of approximately 58kDa in DU145 cells, whilst in HEK 293 cells a tight doublet of this mobility was
10 detected. These bands were not observed upon Western blotting with pre-immune serum or in the cell lines which do not express Grb14 mRNA. This estimated size of Grb14 upon SDS-PAGE is in accordance with the predicted size of the translation product of the Grb14 cDNA (60kDa).

Since DU145 cells overexpress Grb14 mRNA relative to the two other
15 prostate carcinoma cell lines examined (Table 1), we investigated whether this was accompanied by an upregulation of Grb14 protein expression. Upon Western blot analysis, Grb14 was clearly detectable in DU145, but not PC3 or LnCaP, cell lysates, indicating that Grb14 protein is overexpressed in this cell line.

20

Phosphorylation of Grb14

In order to characterize further the role of Grb14 in receptor tyrosine kinase signalling, the phosphorylation state of Grb14 was investigated before and after growth factor stimulation. Since the anti-Grb14 antiserum 264 did
25 not immunoprecipitate Grb14 under either native or denaturing conditions, we utilized an expression construct (pRcCMV_{Flag}) which tagged Grb14 with the 8 amino acid Flag epitope at the C-terminus. This construct was stably transfected into HEK 293 cells, leading to the isolation of stable clones of cells expressing an epitope-tagged Grb14 which could be
30 immunoprecipitated with the M2 anti-Flag monoclonal antibody and Western blotted with either this antibody or anti-Grb14 antiserum 264. Immunoprecipitation of Grb14 from serum starved cells which were metabolically labelled with ³²P-orthophosphate demonstrated that Grb14 was phosphorylated in this basal state. Phosphoamino acid analysis of the
35 isolated protein demonstrated that phosphorylation was on serine residues.

Treatment of the cells with EGF did not significantly increase this level of phosphorylation, although activation of native EGFRs could be demonstrated by anti-phosphotyrosine blotting of the cell lysates. However, stimulation with PGDF BB resulted in an approximately 1.5 fold increase within 5 min of administration, and transient transfection of a cDNA encoding β -PDGFRs into the cells further amplified this response to approximately 2-fold. The small increase in phosphorylation which occurred when this construct was present in the absence of PGDF BB was presumably due to the constitutive activation of RTKs often observed with this system. Phosphoamino acid analysis demonstrated that the PDGF-induced increases in Grb14 phosphorylation also occurred on serine residues.

As will be recognised by persons skilled in this field the present inventors have identified a novel signalling molecule which they have designated GDU or Grb14. GDU has the potential to be used as a prognostic indicator/tumour marker in both breast and prostate cancer. In addition, as GDU may influence invasive/ metastatic behaviour it may also serve as a marker of invasive/metastatic disease in these and other cancers. Finally, the involvement of GDU in signalling by the PDGFR suggests that it may represent a therapeutic target in diseases or conditions in which PDGF plays a regulatory role.

Signalling via GDU could be targeted by competitive peptides or dominant negative mutants, or restored by gene therapy. The information provided herein will clearly assist in the rational design of a GDU antagonist.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

Claims:

1. A polynucleotide encoding GDU, the polynucleotide having a sequence which encodes a polypeptide having an amino acid sequence as shown in Figure 2 or a sequence which hybridises thereto.
- 5 2. A polynucleotide as claimed in claim 1 in which the polynucleotide has a sequence as shown in Figure 2.
3. A GDU polypeptide, the polypeptide having an amino acid sequence as shown in Figure 2.
4. An antibody which binds to the polypeptide as claimed in claim 3.
- 10 5. An antibody as claimed in claim 4 in which the antibody is a monoclonal antibody.
6. An oligonucleotide probe of at least 12 nucleotides, the oligonucleotide probe having a sequence such that the probe selectively hybridises to the polynucleotide as claimed in claim 1 under stringent
- 15 conditions.
7. An oligonucleotide probe as claimed in claim 6 in which the oligonucleotide probe is labelled.
8. An oligonucleotide probe as claimed in claim 6 or claim 7 in which the oligonucleotide is of at least 18 nucleotides.
- 20 9. A method of detecting the presence of GDU in a sample, the method comprising reacting the sample with an antibody as claimed in claim 4 or claim 5 or an oligonucleotide probe as claimed in any one of claims 6 to 8 and detecting the binding of the antibody or the probe.

1/3

Figure 1

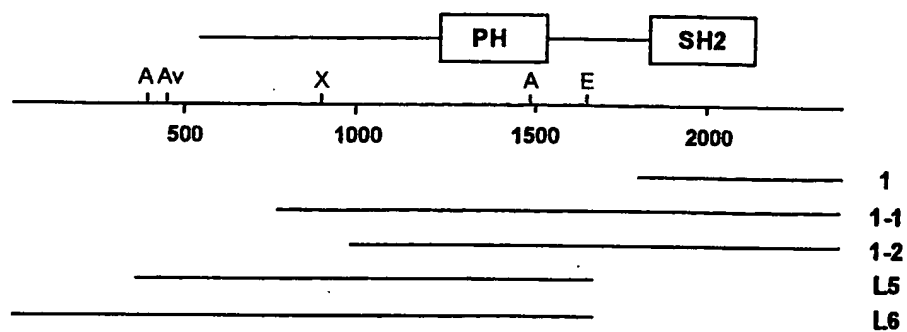


Figure 2

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CGGATGAGGGTCAGGGCTGCGCGGACCCCTATCCCGCCTGCGTCTCCCGGCAAGCCCAGCGGGAGCGCC 70
 CGCTCGGCTGGGTCCCCGCTCCAGCGCGCCGGGGCCGCCCAGACCCTGGGCTCAGCCTCGCGCCCCGGT 140
 GCGGACCTGAGGAGGCGCGGTCCCGGCTCGCGTCCCGGATGGGACGGCGCGGGAGCAATGCCAGTGGC 210
 CCGGAGCGCCCCGGGCCAGCGCGGGGGCCGCGCCAGCCGCTCTCGCGCCCTCCCGCCCCCTCCGCGCCTT 280
 GCCTCGCGCGCGCGCGCCCCACCCACCGCGCGCTCCTCCCTCTCCCGACCCCTCCTCCTCGCCCCCTC 350
 CCCTCCCCCGCGCCTCGCAGATAGCTCGGCGCGCGTCTCAGCCGCGGGGCCCCGAGCGCAGGCGGGC 420
 AGGCCACACACCTGCAGAGCGCTCGGGCTGCCTAGGCGGCACCTCGCCTCCCGCGCGCAAAACCCCTTC 490
 TCCCCACGCGCGAGTCTCCCATGACGCGCGAGCCCCCGCGCGGCGACAATGACCACTTCCCTGCAAGA 560
 M T T S L Q D
 TGGGCAGAGCGCGCGAGCAGGGCGGCTGCCCCGGATTGCGCGCTGGCGCGCCAGGTGTGTGGCGCTGCC 630
 G Q S A A S R A A A R D S P L A A Q V C G A A
 CAGGGGAGGGGCGACGCCCACGACCTGGCGCGGCCCCCTGGCTGCACGCGCGAGCGCTCCTGCCCTTC 700
 Q G R G D A H D L A P A P W L H A R A L L P L
 CGGACGGGACCCGCGGTGTGCTGCAGACAGGAGAAAAAGAAAGATCTTGATGTTCCGGAATGCCATC 770
 P D G T R G C A A D R R K K K D L D V P E M P S
 TATTCCAAACCTTTTCTGAGCTATGCTGTTCTGCAATTACATCTGTGTGTCAGCAGACCTATTCCC 840
 I P N P F P E L C C S P I T S V L S A D L F P
 AAAGCAAATTCAGGAAAAAACAGGTGATTAAAGTATACAGTGAAGATGAAACCAGCAGGGCTTTAGATG 910
 K A N S R K K Q V I K V Y S E D E T S R A L D
 TACCACTGACATAACGGCTCGAGATGTTTGTGAGCTGTGATCCTGAAGAATCATTACATTGATGACCA 980
 V P S D I T A R D V C Q L L I L K N H Y I D D H
 CAGCTGGACCCCTTTTGTGACACCTGCCTCACATAGGTGTAGAAAGAACAATAGAAGACCACGAACCTGGTG 1050
 S W T L F E H L P H I G V E R T I E D H E L V
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 L H A K E Q G K K S W K K I Y F F L R R S G L
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 Y F S T K G T S K E P R H L Q F F S E F G N S D
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 R L I I Q Q G L V D G V F L V R D S Q S N P K
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 AAAAAAAAAAAAAAAAAAAAAA 2404

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Gdb14	1	MTTSLQDQGSAASRAAARDSP	LAAQVCGAAQGRGDAHDLAPAPWLHA	47			
Gdb7	1	MELDLSPTHLSSSPE	-----DVCPTPATPPETPPPPDNP	34			
Gdb10	1	MNNDINSSVESLNSACNMQSDTD	TAPLLEDGQHASNQGAASSSRGQP	47			

Gdb14	48	RALLPLPDGTRGCAAD--RR--	---KKKDLDPVEMPSP	IPNPFPEL	85		
Gdb7	35	---PPGDVKRSQPLPIPSSRKL	REEEFQATSLPS	IPNPFPEL	CS--	75	
Gdb10	48	QAS-PRQKMR	SQPVHILRRRLQEEDQQLRTASL	PAIPNPFPEL	TGAA	93	

Gdb14	86	-----	-----C--CS-----	88			
Gdb7	76	-----	PPSQKP-----	ILGGSSGARG-----	91		
Gdb10	94	PGSPPSVAPSSLP	PPPSQPPAKHCGRCEKWIPGENTRGN	GKRKIWRW	140		

Gdb14	89	-----PITSVLSAD	LFPKANSR--KKQV-----	109			
Gdb7	92	-----	LLPRDSSRL-----CV-----	102			
Gdb10	141	QFPPGFQLSKLTRPG	LWTKTTAR	FSKKQPKNQCP	TDVNPVARMPTS	187	

Gdb14	110	-----	IKVYSEDETSRALDVP	SDITARDV	QQLLILKNHYIDD	146	
Gdb7	103	-----	IKVYSEDEGACRSVEVAAGAT	ARHVC	EMLVQRAHALSD	139	
Gdb10	188	QMEKLRRLRKDV	VKVFSEDEGTSKVVEIL	TDMTARD	LCQLLVYKSHCVD	234	
F10E9.6	187	-----	VKFFVEDGEALQLLIDERW	TVADTLKQLAEKNHIALM	232		

Gdb14	147	HSWTLEFHLPHIGVERTIEDHE	LVIEVLSNWGIEEEN	KLYFRKNYAK	193		
Gdb7	140	ESWGLVESHPYLAALERGLE	DFVVEVQEA	MPVGGDSRFIFRKNFAK	186		
Gdb10	235	NSWTLVEHHPQLGLERCL	EDHEIVVQVEST--	MPSESKFLFRKNYAK	279		
F10E9.6	233	EDHCIVEEYPELYIKRVY	EDHEKVVENIQMWVQD	SPNKLFLMRRPDK	279		

Gdb14	194	YEEFKNP	MY--FFPEH	MVSFATETNGEISPTQIL	QMFLS-----SST	233	
Gdb7	187	YELFKSP	PHTLFPEK	MVSSCLDAQITGISHEDLI	QNFN-----AGS	227	
Gdb10	280	YEEFKNP	PNV--FEPDQ	MVNWCOQSNQ--	GQAQLLQNFN-----TSS	317	
F10E9.6	280	YALFISRP	ELYLLTPKTS	DHMEIPSGDQWTIDVK	KKEFVSEYFHREP	326	

Gdb14	234	YPEIHGFLHAK	EQGK---KSWKKIYF	FLRRSGLYFSTKGTSKE	EPRH	276	
Gdb7	228	FPEIQGFLQLRGS	GRGSGRKLWKRFFC	FLRRSGLYYSTKGTSKE	EPRH	274	
Gdb10	318	CPEIQGFLQVKEVGR	---KSWKKLYVCLRRSGLYYSTKGTSKE	EPRH	360		
F10E9.6	327	PPEIMEGFLYLKSDGR	---KSWKKHYFVLRPSGLYYAPKSKKPTTKD	369			

Gdb14	277	LQFFSEFGNSDIYVSLAG	KKKHGAPTNYGFC	FKPNKA--GGPRDL--KM	321		
Gdb7	275	LQYVADVNESNVYVVTQGR	KLYGMPIDFGFC	VKPNKLRNGHKGL--HI	320		
Gdb10	361	LQLLADLEESSIFYLIAG	KKQYNAPNEHGMCI	KPNKAKTEMKEI--RL	406		
F10E9.6	370	LTCLMNLHNSQVYTGIGWE	KKYKSPTPWCISIKL	TALQMKRSQFIKY	416		

Gdb14	322	LCAEEEQS	RTCWVTAIRLLKYGMQLYQNYMHPYQGR	BSGC--SSQSIS	366		
Gdb7	321	FCSEDEQS	RTCWLAARLLFKYGVOLYKNYQQAQSRHLRL--SYLGSP	365			
Gdb10	407	LCAEDEQ	IRTCWMTAFRLKYGMQLYQNYRIP--QRKGL--PPPFNA	449			
F10E9.6	417	ICAEDMTFKKWLVALRI	AKNGAELLNENYERACQIR	RETLPASSMS	463		

Gdb14	367	PMRSISE	NSLVAMDFSGQK-----SRVIE	NPTEALSVAVE	401		
Gdb7	366	PLRSVSDN	TLVAMDFSGHA-----GRVID	NPREALSAAAME	400		
Gdb10	450	PMRSVSE	NSLVAMDFSGQI-----GRVID	NPAEASAALE	484		
F10E9.6	464	AASSSTAISELVPHSL	SHHQRTPSVASSIQLSSHMMN	NPTHPLSVNV--	509		

Gdb14	402	EGLAWRKKGCLR	LGTHGSPTASSQSSATNMAI	IHRSQPWFFHKKISRDE	448		
Gdb7	401	EAGAWRKKTNHRLSL	---PTTCSSSLSAAI	IHRTPWFHGRISREE	443		
Gdb10	485	EGHAWRN--GSTBMN	ILSSQSPL--HPSTLNAV	IHRTPWFHGRISREE	529		
F10E9.6	510	-----RNQSPAS	FSVNSCQQ--SHPSRTSAKL	534			

Gdb14	449	AQRLLII	IQQGLVDGVFLVRDSQS	NPKTFFVLSMSHGQKIKHFQIIPVED	495		
Gdb7	444	SQRLLIG	QQGLVDGVFLVRESQRNPQG	FVLSLCHLQKVKKHILIPSED	490		
Gdb10	530	SHRIII	KQQGLVDGFLFLVRDSQS	NPKAFVLTLC	HHQKIKNFQILIPCED	576	

Gdb14	496	DGEMFHTLD	DGHTTRFTDLIQLV	EYQLNKGVLPC	KLKHYCARIAL	540	
Gdb7	491	EGCLYFSMDEG	QTRFTDLIQLV	EYHQLNRGILPC	LLRHCCARVAL	535	
Gdb10	577	DGQTFFTLD	DGNTKESDI	IQLVDEYQLNKGVLPC	KLKHHCI	IRVAL	621

FIGURE 3

INTERNATIONAL SEARCH REPORT

International Application No.

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A. CLASSIFICATION OF SUBJECT MATTER												
Int Cl ⁶ : C12N 15/12; C07K 14/47												
According to International Patent Classification (IPC) or to both national classification and IPC												
B. FIELDS SEARCHED												
Minimum documentation searched (classification system followed by classification symbols) IPC : C07K 14/-, C07K 15/-, C12N 15/-												
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched AU IPC : As Above												
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Chem Abs (STN) : Sequence Search WPAT : IPC + (GDU OR ERB # OR EGF OR EPIDERMAL () GROWTH () FACTOR #) + (RECEPTOR () TYROSINE () KINASE # OR RTK # OR SH # () DOMAIN)												
C. DOCUMENTS CONSIDERED TO BE RELEVANT												
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.										
A	Oncogene (1995), 10 (8), pp 1621-30, "The cloning of Grb 10 reveals a new family of SH2 domain proteins", Ooi, J <u>et al</u>											
A	Proceedings of the National Academy of Science, USA (1995), 92 (22) PP 10287-91, "Grb-IR : A SH2-domain-containing protein that binds to the insulin receptor and inhibits its function", Liu and Roth											
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C <input checked="" type="checkbox"/> See patent family annex												
<p>* Special categories of cited documents:</p> <table border="0"> <tr> <td>"A" document defining the general state of the art which is not considered to be of particular relevance</td> <td>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>"E" earlier document but published on or after the international filing date</td> <td>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>"O" document referring to an oral disclosure, use, exhibition or other means</td> <td>"&" document member of the same patent family</td> </tr> <tr> <td>"P" document published prior to the international filing date but later than the priority date claimed</td> <td></td> </tr> </table>			"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	"E" earlier document but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family	"P" document published prior to the international filing date but later than the priority date claimed	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention											
"E" earlier document but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone											
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art											
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family											
"P" document published prior to the international filing date but later than the priority date claimed												
Date of the actual completion of the international search 26 June 1996		Date of mailing of the international search report 4TH July 1996.										
Name and mailing address of the ISA/AU AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (06) 285 3929		Authorized officer BARRY SPENCER Telephone No.: (06) 283 2284										

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/AU 96/00258

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	AU, A, 21022/95 (NEW YORK UNIVERSITY) 25 September 1995	
A	AU, A, 19809/95 (NEW YORK UNIVERSITY MEDICAL CENTRE) 25 September 1995	
A	AU, A, 53633/94 (LUDWIG INSTITUTE FOR CANCER RESEARCH) 24 May 1994	
A	AU, A, 51804/93 (BRISTOL-MYERS SQUIBB COMPANY) 9 June 1994	
A	AU, B, 25185/92 (669857) (MAX-PLANC-GESELLSCHAFT ZUR FORDERUNG DER WISSENSCHAFTEN E.V.) 5 April 1993	
A	Proceedings of the National Academy of Science, USA (1992) 89(19) pp 8894-8, "High-efficiency expression/cloning of epidermal growth factor-receptor-binding proteins with Src homology 2 domains", Margolis <u>et al</u>	